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## Going west: the spread of non-native *Microporella* (Bryozoa) species on drift plastic debris

# Antonietta ROSSO<sup>1,2</sup>, Emanuela DI MARTINO<sup>1</sup>, and Chiara SIDDIOLO<sup>1,3</sup>

<sup>1</sup> Department of Biological, Geological and Environmental Sciences, University of Catania, Catania, Italy

<sup>2</sup> CoNISMa – Inter-University Consortium for Marine Science, Roma, Italy

<sup>3</sup> University School for Advanced Studies IUSS Pavia, Pavia, Italy

Corresponding author: Antonietta ROSSO; rosso@unict.it

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## **Abstract**

Despite being a major threat to biodiversity, often causing homogenization and banalization in ecosystems, the understanding of marine bioinvasions remains limited, especially regarding non-economically important taxonomic groups and newly emerging vectors or dispersion pathways. In this paper, we report the finding of viable colonies of five *Microporella* species on plastic debris stranded on sandy beaches, predominantly in the Ionian Sea, and subordinately in the Tyrrhenian Sea and Sicily Strait. Three species, *M. appendiculata*, *M. modesta* and *M. ichnusae*, are native to the Mediterranean, with the former two widespread in the investigated area, and the latter previously known only from its type locality in northwestern Sardinia, now widening its known distribution range to the west coast of Sicily. Conversely, findings of *M. hastingsae* and *M. browni* in Sicily represent localities far from their previously known distributions in the Suez Canal and the Levantine and Red Sea-Indian Ocean regions, respectively. We suggest that these pioneering colonies, found on drift plastics between April 2023 and May 2024, may have arrived through rafting by surface currents, at least in the western Ionian Sea. Human activities, especially commercial and recreational shipping, may have also facilitated their spread, especially in the Tyrrhenian Sea and the Sicily Strait. Fouling on buoyant plastics could originate directly from unknown populations in the eastern Mediterranean, or secondarily through shipping, which may have introduced larvae that subsequently attached to plastics along the coast of Sicily. These findings point to the need for further investigations to confirm whether established populations of *M. hastingsae* and *M. browni* exist in coastal artificial and/or natural habitats in these areas.

Keywords: Distribution range; Lessepsian species; fouling communities; taxonomic remarks; Sicily; Mediterranean.

## Introduction

The ongoing introduction of Non-Indigenous Species (NIS) is a significant and increasing threat to global biodiversity, including in the Mediterranean (e.g., Tsirintanis et al., 2022; Zenetos et al., 2022a). This consideration extends to bryozoans, with a continuous increase in species records (e.g., Katsanevakis et al., 2020; Ragkousis et al., 2023) and new species becoming established (Zenetos et al., 2022b). While shipping and aquaculture are well-recognised pathways for NIS introduction in the Mediterranean (e.g., Zenetos et al., 2012), the role of drift plastic and other waste materials has not been thoroughly examined. Roura-Pascual et al. (2021) did not account for the impact of waste production/reduction/removal on generating drift plastics in their future scenario analysis for global biological invasions, despite focusing on socioecological drivers, such as "lifestyle and recreation" or "social norms and values", which could encompass these factors. However, plastic debris is colonised by various species, especially opportunistic and non-indigenous ones that can establish dense, specialised communities, with shallow-water filter-feeding invertebrates particularly adapted and advantaged (e.g., Kiessling *et al.*, 2015). Moreover, since the introduction of plastics approximately a century ago, the volume of anthropogenic drift material has surged, with its buoyancy enabling long-distance transport via surface currents, increasing the risk of species dispersal.

Despite some exceptions, the potential of long-lasting and long-distance transport (e.g., Murray *et al.*, 2018) and the role of rafting on drift material in species spread (e.g., Winston *et al.*, 1997; Lewis *et al.*, 2005; Audrézet *et al.*, 2021; Kannan *et al.*, 2023), has likely been underestimated especially in the Mediterranean Sea. Similarly, the contribution of these substrata to local transfer of non-native species (NNS) remains underexplored, although plastic panels and nets have proven effectiveness

in recruiting and could be useful for monitoring first arrivals of new species (e.g., Miralles *et al.*, 2018; Giacobbe *et al.*, 2024).

In this context, we document the presence of two NNS of the genus Microporella found on plastic litter beached in several localities along the coast of Sicily. Microporella browni Harmelin, Ostrovsky, Cáceres-Chamizo & Sanner, 2011 is first reported from locations outside the coast of Lebanon, where it was previously known within the Mediterranean. We also confirm the occurrence of M. hastingsae Harmelin, Ostrovsky, Cáceres-Chamizo & Sanner, 2011 in the western Ionian Sea after the discovery of a first colony on plastic waste collected at the mouth of the Simeto River, along the Plaia, south of Catania, in April 2023 by Rosso & Siddiolo (2024). We provide information about morphological variability and discuss potential pathways for the transport of these species from their previously known distribution areas in the Levantine Sea and the Suez Canal, respectively.

### **Materials and Methods**

The material examined was collected after some surveys conducted in the period between April 2023 and May 2024 across various coastal locations (sandy beaches) in Sicily (Fig. 1). Except for Magaggiari beach near Palermo, located in the SW Tyrrhenian Sea, the remain-

ing sites are located along the eastern coastline of the island, ranging from the Capo Milazzo Peninsula projecting into the southeastern Tyrrhenian Sea to Santa Maria del Focallo beach in the very eastern part of the Sicily Strait, including localities around the Messina Strait and near Catania in the western Ionian Sea. The names and coordinates of these localities are listed in Table 1.

The surveys were part of a monitoring project focused on fouling communities found on drift items that usually wash ashore after storms. The material was collected either by picking encrusted plastic debris during 30- or 45-minutes walks or collecting all plastic debris along 100-meter-long by 1-meter-wide transects roughly parallel to the coastline. The stranded material predominantly consisted of plastic items varying in nature, size and colour. Upon visual inspection, many of these items revealed the occurrence of fouling communities, often including bryozoans. For this study, we selected all items containing colonies of Microporella species. Bryozoans were examined under a stereomicroscope, and selected *Micro*porella colonies were photographed, untreated and uncoated, using a Tescan Vega 2 LMU, Low Vacuum Scanning Electron Microscope (SEM) at the Microscopical Laboratory of the Department of Biological, Geological and Environmental Sciences of the University of Catania (DSBGA). Images were generated using back-scattered electrons. Measurements were taken from SEM micrographs using the image processing program ImageJ

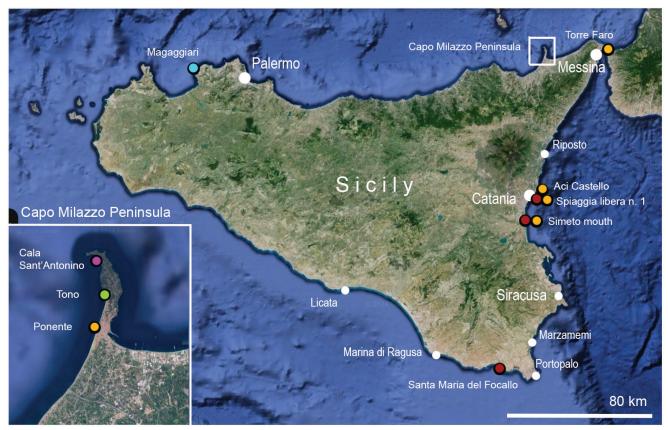


Fig. 1: Map of Sicily with an inset showing the Capo Milazzo Peninsula (sourced from Google Earth), highlighting the survey locations from 2023 and 2024 where Microporella species were recorded. Microporella browni: yellow; M. hastingsae: red; M. appendiculata; green; M. ichnusae: pale blue; M. modesta: purple. Main towns/cities either near the surveyed beaches or simply cited in the text are indicated by white small and large dots, respectively.

**Table 1.** List of surveys for stranded items that delivered species of *Microporella*. *M.b.*: *Microporella browni*; *M.h.*: *Microporella hastingsae*; *M.a.*: *Microporella appendiculata*; *M.i.*: *Microporella ichnusae*; *M.m.*: *Microporella modesta*. For each survey, the date, sampling method, name of the locality (including information about the sea sector and province), coordinates, and the specific type of item on which each species was found are indicated. For picking surveys only the coordinates of the starting point are provided. The § symbol indicates the colony already studied in Rosso & Siddiolo (2024); the number of asterisks is indicative of the abundance of colonies: \*=1 colony; \*\*= few colonies; \*\*\*= several colonies.

Date	Sampling method	Sea	Province	Beach site	Coordinates	Substratum	M.br.	M.h.	М.а.	M.i.	М.т.
Dec. 2023	Picking	Tyrrhenian Sea	Palermo	Cinisi, Magaggiari	38.15965N; 13.08397E	Plastic container				**	
Apr. 2024	Picking		Messina	Milazzo, Ponente	38.21959N; 15.23237E	Unidentified plastic item	*				
May 2024	Picking			Milazzo, Tono	38.24508N; 15.24111E	Unidentified thin plastic sheet			*		
Apr. 2024	Picking			Milazzo, Cala Sant'Antonino	38.26446N; 15.23690E	Plastic water bottle					*
Apr. 2024	Picking			NE to the city, Torre Faro	38.26744N; 15.65332E	Plastic bottle cap	*				
Apr. 2024	Picking		Catania	Acicastello, N to the castle	37.55584N; 15.14848E	Plastic water bottle	*				
Sept. 2023	Picking			Plaia, Spiaggia libera n. 1	37.48549N; 15.08713E	Lifejacket strap	*				
Apr. 2024	Transect			Plaia, Spiaggia libera n. 1	37.48615N; 15.08869E 37.48686N; 15.08951E	Bivalves on lifejacket, plastic buckles and nylon fabric	***	**			
Apr. 2023	Picking			Plaia, Simeto mouth	37.39091N; 15.09024E	Plastic fruit box		§			
						Bouy	***				
Apr. 2024	Picking	Sicily Strait	Ragusa	Santa Maria del Focallo	36.72901N; 14.89777E	Plastic container lid		**			

(available from https://imagej.nih.gov/) and are given in the text as mean values, when applicable followed by the total range and the number of measurements, in brackets. Abbreviations for the measurements are: O, orifice; Ov, ovicell; L, length; W, width.

The studied material is housed in the Paleontological Museum (PMC) of the DSBGA, in the Rosso and Di Martino collection, under the catalogue numbers PMC AR-EDM Collection I. H. B.111a, a1 and b for *M. hastingsae*, from Plaia (Catania) 2023 and 2024 and Santa Maria del Focallo beaches, respectively; PMC AR-EDM Collection I. H. B.117a, a1, a2, b and c, for *M. browni* from Plaia (Catania) 2023, 2024, Aci Castello, Ponente (Milazzo, Messina) and Torre Faro (Messina), respectively; PMC AR-EDM-Collection I. H. B.84f for *M. appendiculata* from Tono (Milazzo, Messina); PMC AR-EDM

Collection I. H. B.118a for *M. ichnusae* from Magaggiari beach (Palermo), and PMC AR-EDM Collection I. H. B.86l for *M. modesta* from Cala Sant'Antonino (Milazzo, Messina).

## Results

Beached litter items, including *Microporella* colonies, were frequently recovered during the surveys conducted within the study period and in several of the surveyed localities (eight out of 17). Only at one of the three sites surveyed in the Palermo province (Magaggiari, Tyrrhenian Sea), were *Microporella* colonies collected in litter samples, whereas colonies occurred in samples of all three sites in the Capo Milazzo Peninsula (Ponente, Tono

and Cala Sant'Antonino, still along the Tyrrhenian coast). Microporella colonies were also found at one of two sites near Messina, i.e., Torre Faro beach on the Messina Strait, but not at the Canalone site, a few kilometres south on the nearby Ionian coast. Also along the Ionian coast, three of the five surveyed sites in Catania province (Acicastello, Spiaggia libera n. 1 and Simeto River mouth) yielded colonies, whereas none were found at the two sites in Siracusa province. Finally, colonies were also found at one (Santa Maria del Focallo) of the two sites investigated along the coast of Ragusa province in Sicily Strait. Although most localities were surveyed only once, those near Catania were visited twice, and at one site (Spiaggia libera n. 1) Microporella colonies were found on both occasions (in September 2023 and April 2024).

Most collected items were made of hard plastic material, such as containers, lids, and bottle caps, as well as an old broken buoy (Fig. 2, Table 1). Notably, in both 2023 and 2024 surveys at Spiaggia libera n. 1, Microporella colonies were found on stranded lifejackets (Fig. 2 A-D).

A total of five *Microporella* species were identified: Microporella appendiculata (Heller, 1867), M. ichnusae Di Martino & Rosso, 2021, M. modesta Di Martino, Taylor & Gordon, 2020, M. hastingsae and M. browni. The first three species were found occasionally, with M. appendiculata and M. modesta yielding only a colony each at only one site, while numerous colonies of M. ichnusae were found at Magaggiari beach. In contrast, the latter two species occurred at four and five sites, respectively (Fig. 1, Table 1). Several colonies of both M. hastingsae and M. browni were found on lifejackets, encrusting different kinds of surfaces, including the hard-plastic buckles (Fig. 2C) and the comparably lighter and more flexible straps (Fig. 2B). In the 2024 sample, both these species were mostly found on the hard secondary substrata provided by the carbonate shells of the oyster bivalve Ostrea stentina Payraudeau, 1826 (Fig. 2D), which colonised all surfaces available on the lifejackets, including the nylon fabric.

The two non-native Microporella species showed different distributions and abundance patterns (Table 1). Microporella hastingsae was predominantly found in southeastern Sicily, particularly around the Gulf of Catania and along the eastern coast of Sicily in the Sicily Strait. In contrast, M. browni was found in the northeastern part of the island, primarily in the Gulf of Catania, extending northward toward the Messina Strait and into the eastern Tyrrhenian Sea. It was usually found with isolated or few colonies, except for the 2023 buoy with numerous young non-ovicellate (infertile) colonies and the 2024 lifejacket with very abundant both young and ovicellate (fertile) colonies.

Except for M. appendiculata, the other species were described within the last dozen years (Harmelin et al., 2011; Di Martino et al., 2020; Di Martino & Rosso, 2021). Some of these species were previously known only from a few colonies, and the present findings allowed for the observation of additional characters, contributing to a better understanding of their intraspecific variability.

### Taxonomic remarks

# Microporella ichnusae Di Martino & Rosso, 2021 (Figs 1, 3)

A relatively large colony and several smaller ones were collected from Magaggiari beach in the southwestern Tyrrhenian Sea. These specimens showed all the typical characters (Fig. 3A-E) of the species recently established from a shallow-water, semidark cave habitat in western Sardinia, and provided the first observation of the ancestrula (Fig. 3F), which is tatiform, 287 µm long by 185 µm wide, with a large opesia measuring 140 µm in length and 112 µm in width. The ancestrula features proximally widely exposed gymnocyst that narrows laterally, surrounding a smooth cryptocystidean band that is wider proximally (ca 35 µm) but tapers off laterally and disappears distally. Gymnocyst and cryptocystidean band are separated by a thin, smooth, raised rim indented by four closely spaced oral spines and an additional five more widely spaced, evenly distributed proximal spines (Fig. 3F). The ancestrula typically buds two distolateral asymmetrical autozooids (Fig. 3G) and is often eccentrically positioned within the colony, remaining marginal in relatively large colonies (Fig. 3F). In one case, the ancestrula was occluded by a tuberculate and perforate cryptocystidean cover, possibly the result of intramural regeneration as a kenozooid (Fig. 3G, H). The rebudding of the ancestrula as a kenozooid was previously observed in M. modesta (Di Martino & Rosso, 2021). Additionally, the ancestrula in one colony of M. ichnusae was largely obscured by extensions of the frontal shield from an irregularly shaped, giant, proximal periancestrular autozooid (Fig. 3G) leaving only a portion of its oral sector visible with three spines still attached (Fig. 3H), whereas only the bases of the other spines pierced the cryptocystidean cover.

# Microporella hastingsae Harmelin, Ostrovsky, Cáceres-Chamizo & Sanner, 2011 (Figs 1, 2, 4, 5)

The colonies (Fig. 4), including juveniles, found on the examined stranded items allowed the first SEM documentation of the ancestrula and periancestrular autozooids for this species. Microporella hastingsae was introduced by Harmelin et al. (2011) for specimens of M. ciliata var. coronata, reported by Hastings (1927) from the Suez Canal (Fig. 5, red triangles), and was subsequently formally described by Rosso & Siddiolo (2024). Notably, the ancestrula was absent in the colony studied by these latter authors and was neither described nor figured by Harmelin et al. (2011). However, the specimen from Hastings' collection at the Natural History Museum in London, which was selected as the holotype of M. hastingsae, did include the ancestrula and periancestrular autozooids, as indicated by Hastings' (1927) rough drawing. The ancestrula (Fig. 4C-G) is tatiform and ovoidal, measuring at least 260-300 µm in length and 210-240 µm in width. It has a large opesia (200-220 μm long by 150-180 μm wide) and a depressed, almost smooth cryptocystidean band proximally developed to about 40 µm. The opesia is outlined by a thin, smooth, raised rim, and a relatively

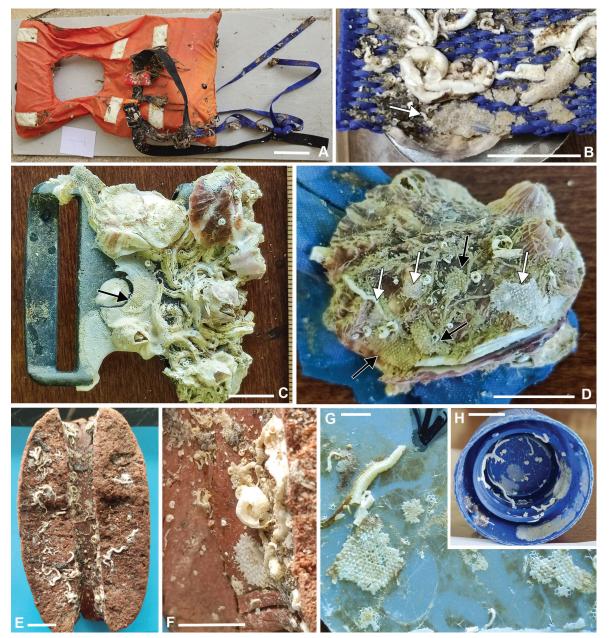


Fig. 2: Selection of substrata where Microporella species were found. (A) Lifejacket stranded at Plaia, south of Catania (Spiaggia libera n. 1), in September 2023, with obvious encrusters mostly on the straps and buckles. (B) An ovicellate colony of M. browni (arrowed) on a strap from the lifejacket in (A). (C) A plastic buckle from the lifejacket stranded at Plaia, south of Catania (Spiaggia libera n. 1) in April 2024. Microporella browni (arrowed) encrusts an almost entirely detached bivalve. (D) Colonies of M. browni (white arrows) and M. hastingsae (black arrows) on the same shell of the bivalve Ostrea stentina, encrusting the plastic fibers of the 2024 lifejacket. (E) and (F) A heavily encrusted broken buoy collected at the Simeto River mouth in April 2023, and close-up showing several young bryozoan colonies, many of which belong to M. browni. (G) A young colony of M. ichnusae between Fenestrulina colonies and serpulids on a plastic container collected in December 2023 at Magaggiari beach (Cinisi, Palermo) (H) Colonies of M. browni on a plastic cap collected at Torre Faro (Messina) in April 2024. Scale bars: 10 cm: (A); 1 cm: (B), (C), (D), (E), (H); 5 mm: (F), (G).

steep gymnocyst that is at least  $100~\mu m$  wide proximally and  $70~\mu m$  wide laterally, when exposed. Nine, occasionally 10 spines occur, unevenly spaced around the opesia, sometimes slightly indenting it. Around the orifice, 4-5 spines are usually slightly less spaced, while the others are more widely spaced in the proximal half, often with two situated at ancestrula mid-length. The ancestrula buds two distolateral autozooids of different size. It may abut or delay budding on one side, leading to asymmetrical colonies (Fig. 4C, F), although it is typically soon over-

grown by autozooids developing in a proximal position (Fig. 4H). Periancestrular autozooids show 5-6 spines (Fig. 4E), a configuration not reported by Hastings (1927) or by Harmelin *et al.* (2011) but within the variability reported by the latter authors for oral spines. According to Hastings' (1927) description, the two autozooids budded from the ancestrula and initial periancestrular ones lack avicularia (Fig. 4C-H). Further autozooids with a single avicularium placed proximolaterally to the ascopore on either side also occur, with a tendency to bud their single

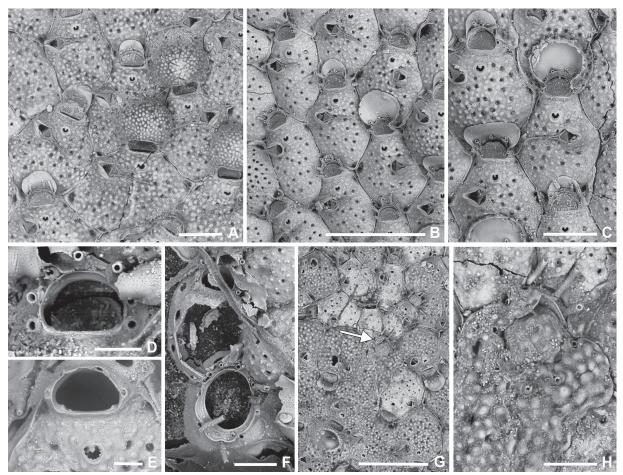


Fig. 3: Microporella ichnusae Di Martino & Rosso, 2021. General features and ancestrula. All images from colonies on a plastic container stranded at Magaggiari beach, Cinisi, Palermo in December 2023. (A) Ovicellate and non-ovicellate autozooids with the typical frontal shield and the different sculpture of the wide ovicells with an imperforate frontal area. (B) Autozooids from a young colony with some forming ovicells. (C) Close-up of ovicells in formation. Note the subcircular finely denticulated ascopore and the minute distal process. (D) and (E) Orifices with an entire, smooth distal margin and shoulder-like proximal condyles. Note the variability in the number of spines and details of the ascopore in (E). (F) Fully exposed ancestrula in an asymmetrically developed colony. (G) Ancestrula (arrowed) and periancestrular autozooids, some lacking the frontal cuticle (lighter grey). (H). Close-up of the ancestrula arrowed in G, largely covered by frontal shield extensions of a proximal autozooid, only leaving three distal spines fully exposed. Scale bars: 200 μm: (A), (C); 500 μm: (B), (G); 50 μm: (D), (E); 100 μm: (F), (H).

avicularium on the right or left side depending on their position relatively to the ancestrula. However, this pattern is not rigid, as indicated by Hastings (1927). With astogeny, the position of the avicularium shifts progressively distally, and two avicularia lateral to the orifice develop (Fig. 4C, D). In some areas, possibly due to damage and irregular growth, some autozooids may fail to develop one or both avicularia (Fig. 4A, B, see asterisks), as previously observed by Rosso & Siddiolo (2024), or may form two proximally shifted avicularia located at level with the ascopore, or two asymmetrical avicularia in different locations in the distal half of the autozooid (Fig. 4B, see black dot). When single, the avicularium may also lack distal orientation, becoming distolaterally oriented. Sometimes, even paired proximally shifted and/ or asymmetrical avicularia tend to diverge from each other, pointing distolaterally rather than distally in parallel.

The absence of distally located paired avicularia in some periancestrular autozooids, or even generations of periancestrular autozooids showing only single, often laterally tilted avicularium (Fig. 4C-F), complicates the

rapid identification of living juvenile colonies. Without the diagnostic ovicells and the pair of latero-oral, distally directed avicularia, young colonies resemble those of other species, particularly *M. browni*, which often co-occurs with several colonies in our samples (Fig. 2D), and the observation of the orifice and further minor characters becomes pivotal.

As noted by Rosso & Siddiolo (2024), regeneration in this species can result in irregularly shaped and oriented autozooids.

# Microporella browni *Harmelin, Ostrovsky,* Cáceres-Chamizo & Sanner, 2011

(Figs 1, 2, 5, 6-10)

Microporella browni was recently described (Harmelin et al., 2011) based on type material collected in 2009, at 9 m depth off south Oman, Red Sea (Fig. 5, yellow triangles), encrusting the shell of the gastropod Haliotis mariae W. Wood, 1828. Further colonies, also ascribed to this species by Harmelin et al. (2011) were found across a wide geographical range in the Indian Ocean, from Oman

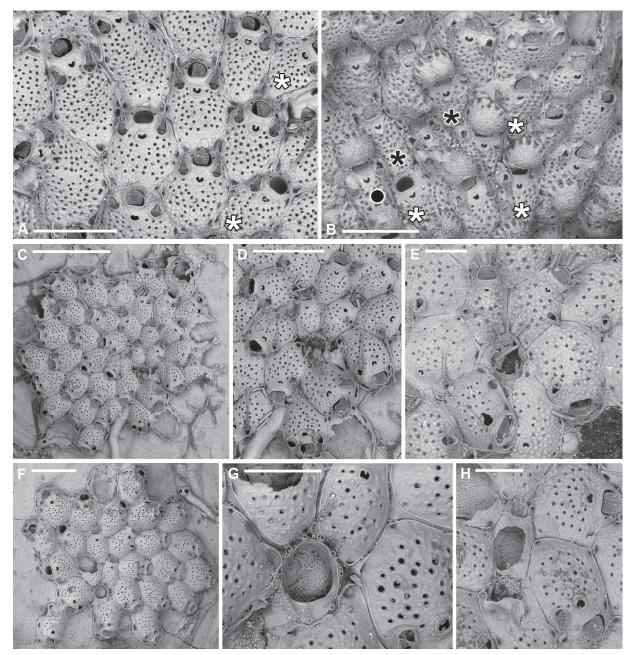


Fig. 4: Microporella hastingsae Harmelin, Ostrovsky, Cáceres-Chamizo & Sanner, 2011. General features, ancestrula and periancestrular autozooids. All images from colonies found on the lifejacket collected in April 2024 at Spiaggia libera n. 1, Plaia, Catania. (A) Non ovicellate autozooids with the typical paired latero-oral avicularia, except for two (see asterisks) having a single avicularium. (B) Autozooids, some with the diagnostic ovicell characterized by large marginal areolae. Note that some autozooids lack one (white asterisks) or both (black asterisks) avicularia or have two avicularia at different levels (black dot). (C) and (D) Young colony (C) and close-up of the ancestrula and periancestrular autozooids (D). Note as most autozooids lack avicularia or show only one avicularium preferentially on the right and left side in zooids budded on the right or left side of the ancestrula, respectively. (E) Ancestrula and periancestrular autozooids with a single avicularium in a very proximal position. Note the length of spines. (F) and (G) Young colony (F) and detail of the ancestrula (G) with detached spines making evident the development of the proximal cryptocystidean band. (H) Ancestrula partly covered by a proximal autozooid, some spines still obvious. Scale bars: 500 μm: (A), (B), (D), (F); 1 mm: (C); 200 μm: (E), (G), (H).

to the Gulf of Aden and the Maldive Islands, in shallow waters between 5 and 19 m depth. These colonies, obtained between the years 1969 and 2009, invariably encrusted the exoskeleton of marine benthic invertebrates, including bivalves and a stony coral. Additional colonies were collected from the Mediterranean off Beirut (Lebanon), in a comparable depth range (3-11 m), including two colonies on serpulid tubes in 2002 and four colonies

on shells in 2003.

Colonies confidently identified as *M. browni* were also found on material stranded on beaches of the eastern Tyrrhenian and the Ionian Seas (Fig. 1, yellow dots; Table 1). The main characters used for their identification were the morphology of the primary orifice and the ovicell.

The primary orifice is transversely semicircular with a denticulate distal margin showing 11-13 relatively



*Fig. 5:* Global known distribution of *Microporella browni* (yellow symbols) and *M. hastingsae* (red symbols). Circles indicate sites from which the new colonies studied here originate, triangles refer to records previously published in Harmelin *et al.* (2011) (i.e., Lebanon and localities outside the Mediterranean) and Rosso & Siddiolo (2024) (i.e., Sicily).

spaced, triangular denticles and a straight proximal margin, with shoulder-shaped condyles at the corners and a corrugated edge in between (Fig. 6C, D, F). The number of distal denticles lowers to 8-10 in periancestrular zooids (Fig. 7B, C, E, H). The presence of a denticulate anter distinguishes M. browni from all other representatives of the genus known to date from Sicily and the western Mediterranean (see Di Martino & Rosso, 2021). Harmelin et al. (2011) remarked that orificial characters are highly diagnostic for this species, despite some variations in the number of denticles (with ranges from 10-13 in the Maldives to 13-18 in the Oman Gulf populations). They also noted the roughness of the proximal margin, which varied among the different populations they studied across these widespread areas. The number of denticles observed in our specimens falls within the total range reported by Harmelin et al. (2011) but is lower than that reported for the Lebanese population in the Mediterranean (12-16 denticles). However, it more closely aligns with the ranges reported for the Red Sea (10-16) and the Maldivian (10-13) populations.

Harmelin et al. (2011) also compared their findings with images provided by Tilbrook (2006), noting that the distinct shape of the orifice, especially the proximal shoulder-like condyles (Fig. 6C, D, F), and the morphology of the avicularian mandible -which is setiform, relatively long, straight and not hooked (Figs 6C; 8E, F)—are crucial for excluding the possibility that our colonies belong to the similar species Microporella orientalis Harmer, 1957, which has been occasionally misreported in the Mediterranean (see also Di Martino & Rosso, 2021). In addition to the differences remarked by Harmelin et al. (2011), M. orientalis also shows a significantly smaller and wider-than-long orifice measuring approximately  $30 \times 60 \mu m$  (OL/OW = 0.50), as reported by Tilbrook (2006). In contrast, our specimens have a larger orifice with mean dimensions of 85 µm (74-93, 12) in length and 111 µm (100-124, 12) in width, resulting in an OL/ OW ratio of 0.77. These measurements are more similar to those reported for M. browni by Harmelin et al. (2011), with orifice dimensions of 72 x 97 μm, 74 x 93 μm and 81 x 117  $\mu$ m (OL/OW ranging from 0.69 to 0.79) in the Oman, Maldives and Red Sea populations, respectively. However, they are slightly longer but narrower than those from Lebanon (78 x 109  $\mu$ m; OL/OW=0.72).

Another important diagnostic character of M. browni is the personate ovicell with a distinctive granular collar that develops distal to the ascopore, encircling the orifice adjoining the proximal wall of the ovicell, exposing a coarsely granular and evenly perforated endooecium that is surrounded by a thin ectooecium (Figs 6A; 8; 9B, G). The elevation of the collar distal to the ascopore creates a tubular structure with two lateral sutures and a single terminal transversally elliptical secondary orifice. This feature sets M. browni apart from M. bicollaris Di Martino & Rosso, 2021, the only other species with personate ovicells reliably reported from Sicily and the western Mediterranean. In M. bicollaris, the collar also embraces the ascopore and, in addition to the secondary orifice, forms a second smaller proximal aperture that connects the ascopore to the ambient water. The personate ovicell of M. browni is also similar to that of M. genisii (Audouin & Savigny, 1826). However, as Harmelin et al. (2011) noted, M. genisii -which they also reported from the Levantine Sea- has peristomes that leave one or two oral spines visible, a feature absent in our colonies. Furthermore, the orifice in M. genisii lacks both a denticulate distal margin and proximal condyles. The occurrence of ovicellate colonies on the lifejacket collected in September 2023 supports the preliminary identification of the exclusively young colonies collected in April 2023 from the buoy at the Simeto River mouth as M. browni. Although the personate collar is a diagnostic feature, it sometimes fails to develop fully. In some ovicells, either clustered together or scattered among others, the collar appears with a completely different aspect (Fig. 8A-E). These ovicells often lack the proximal part of the collar, which instead develops only two lateral wings that join and suture laterally to the proximal side of the ovicell, consisting of a folded rim of gymnocystal calcification appearing somewhat continuous with the ectooecium (Fig. 8C). Although the incomplete development of the collar, and especially of its proximal part, has not been previously reported for M. browni by Harmelin et al. (2011), it seems to be

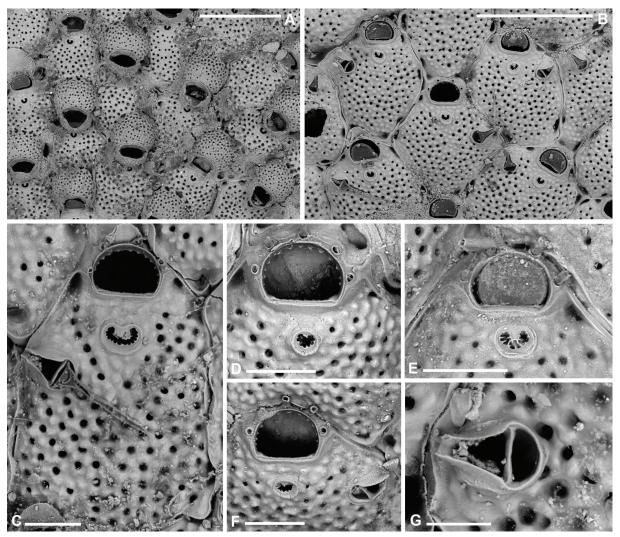


Fig. 6: Microporella browni Harmelin, Ostrovsky, Cáceres-Chamizo & Sanner, 2011. Main diagnostic characters. Colonies found on the lifejacket collected in April 2024 at Spiaggia libera n. 1, Plaia, Catania (A-C and G), on a different lifejacket found at the same locality in September 2023 (E), and on a buoy stranded at the Simeto River mouth in April 2023 (D and F). (A) Cluster of "personate" ovicells most with the typical peristome developed distally to the ascopore. (B) Non ovicellate autozooids. Note the local consistent presence of the frontal avicularium with the long and brittle setiform mandible. (C) An autozooid. Note the reniform ascopore field with large subrectangular distal process and the straight setiform mandible. (D), (E), (F) Details of some autozooids' distal half showing the variability of the orifice, number of oral spines and morphology of the ascopore sometimes with anastomosing denticles forming bars. (G) Close-up of an avicularium. Scale bars: 500 μm: (A), (B); 100 μm: (C), (D), (E), (F); 50 μm: (G).

a relatively common condition in personate ovicells of at least some *Microporella* species. It has occasionally been observed in *M. genisii* from Safaga Bay, Red Sea, *M. harmeri* Hayward, 1988 from South Africa, and *M. maldiviensis* Harmelin, Ostrovsky, Cáceres-Chamizo & Sanner, 2011 from the Maldive Islands, and also in the holotype and paratype of *M. collaroides* Harmelin, Ostrovsky, Cáceres-Chamizo & Sanner, 2011 from Jeddah, Red Sea (Harmelin *et al.*, 2011). In our specimens, some ovicells, especially those with incomplete collars, show a less nodular frontal surface and even a texture of fine, pointed granules (Fig. 8C, D, G).

Ovicells in our specimens have a mean length of 211  $\mu$ m (189-230, 15) and a mean width of 271  $\mu$ m (251-291, 15), resulting in an OvL/OvW ratio of 0.78. These measurements largely overlap with those reported by Harmelin *et al.* (2011) for colonies from Lebanon (211  $\mu$ m x

249  $\mu$ m; OvL/OvW=0.85), and they also fall within the total range reported in their table 1, but seem relatively different –being longer and narrower– compared to the measurements from the type locality, which are 170  $\mu$ m long by 256  $\mu$ m wide (OvL/OvW=0.66).

Further slight variability was observed in the frontal, moderately convex shield of the polygonal autozooids, which is pierced by a highly variable but relevant number of pseudopores, ranging from 56 to 118 in the examined autozooids from the zone of astogenetic repetition. This number largely overlaps with the total range of 31-98 pseudopores observed by Harmelin *et al.* (2011) but is shifted towards higher values, even exceeding the 70-95 and the 93-98 pseudopores observed in the Omani and Lebanese populations, respectively. This high number of pseudopores seems related to the dimensions of the frontal surface, which is wider in zooids collected on

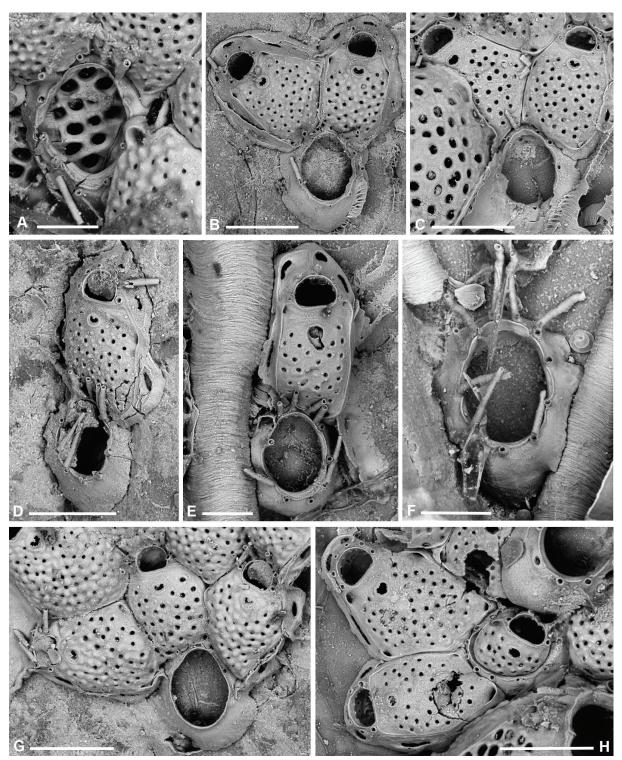


Fig. 7: Microporella browni Harmelin, Ostrovsky, Cáceres-Chamizo & Sanner, 2011. Ancestrula: note the gymnocyst lacking any pore indicating future budding loci. All images from colonies found on a buoy stranded at the Simeto River mouth, Plaia, Catania, in April 2023. (A) Ancestrula of a young asymmetrical colony. Note the distal denticulation. (B) Ancestrula with the two first budded distolateral zooids. (C) Ancestrula with detached spines, some bases already covered. (D) and (E) Very young stages with only one budded distolateral zooid, the second one possibly prevented/delayed by the substrate irregularities or further environmental drivers. Note the distal denticulation in (E). (F) Ancestrula without any budded zooid, confidently attributed to M. browni because of its peculiar distal denticulation and the presence on the same substrate of several young colonies of the same species. Note the 11 long spines. (G) Teratological ancestrula with only four distal spines. (H) Ancestrula regenerated as a miniature zooid with almost reverse polarity. Scale bars: 100 μm: (A), (E), (F); 200 μm: (B), (C), (D), (G), (H).

stranded plastics in Sicily (measuring 546  $\mu$ m [454-630, 20] x 384  $\mu$ m [284-530, 20]) than in other populations of this species studied by Harmelin *et al.* (2011). The largest

zooidal dimensions were observed in the Lebanon and Red Sea colonies, measuring 509  $\mu m$  x 384  $\mu m$  and 511  $\mu m$  x 364  $\mu m$ , respectively. Pseudopores are consistently

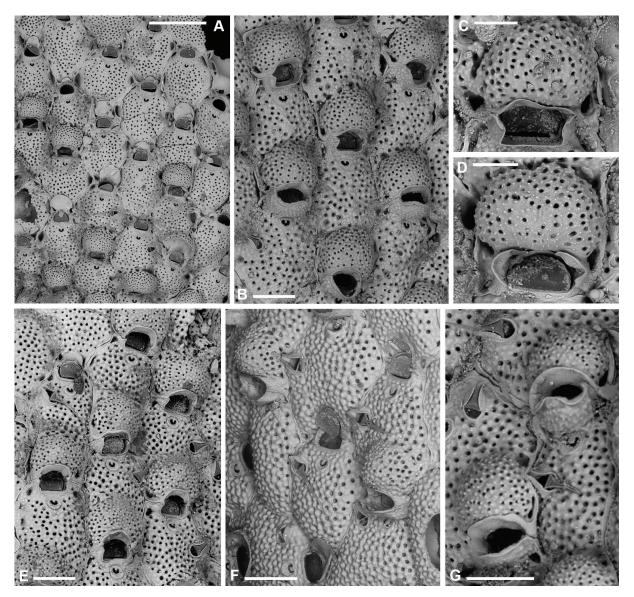


Fig. 8: Microporella browni Harmelin, Ostrovsky, Cáceres-Chamizo & Sanner, 2011. Ovicell variability. All images from colonies found on the lifejacket collected in April 2024 at Spiaggia libera n. 1, Plaia, Catania, except for (F) originating from a plastic cup found at Torre Faro, near Messina in April 2024. (A) Transition between ovicellate and non-ovicellate sectors. Note that most autozooids lack avicularia. (B) Autozooids rarely showing frontal avicularia and ovicells with differently developed peristomes. (C) and (D) Young ovicells with incomplete peristomes and finely granular surfaces. (E) Group of ovicells lacking the proximal peristome. (F) and (G) Ovicells with fully developed peristomes and coarsely granular frontal surfaces. Scale bars: 500 μm: (A); 200 μm: (B), (E), (F), (G); 100 μm: (C), (D).

absent from the suboral area, both distal and lateral to the ascopore (e.g., Fig. 6A-F), but they display a variable distribution pattern on the rest of the frontal shield, either being evenly distributed or more variably clustered, leaving imperforate, irregularly shaped, variably located patches usually around the ascopore and proximal to it in a longitudinally elongate area, or some peripheral zones (Figs 6B; 8A, B). Imperforate patches are especially common in teratological autozooids (Fig. 9A, E-G). A somewhat similar variability, although not as relevant, has been documented between zooids of colonies examined by Harmelin *et al.* (2011: figs 1B; 2A, B) from different geographical areas. Finally, in colonies from Sicily, the ascopore field is variably elevated, though it is consistently less elevated than in the material studied by

Harmelin et al. (2011).

Similar variability in several zooidal features has been observed both in our colonies and by Harmelin *et al.* (2011). For instance, the spiny processes of the ascopore that can occasionally fuse to form bars (Fig. 6E). The number of oral spines also varies, being more numerous (usually 5-6) in the periancestrular autozooids (Fig. 7B, E, G, H), but decreasing to four or even three in later autozooids (e.g., Fig. 6B-F). Additionally, some autozooids may lack adventitious avicularia entirely (Figs 8A, B, E; 9I), mostly the periancestrular ones (Fig. 7B-E, G, H), while others may even develop paired avicularia occasionally (Fig. 9B, C). This peculiarity was previously observed by Harmelin *et al.* (2011) in colonies from the Red Sea and was allegedly considered indicative of

a local morphotype. The occurrence of paired avicularia in our colonies from Sicily challenges this assumption, because they share some characters with the Red Sea colonies but more closely resemble those from Lebanon in other features.

The ancestrula was described as tatiform with 10-11 spines (Harmelin *et al.*, 2011), which is also the common appearance observed in our material (Fig. 7A-F), including a group of 5-6 more closely spaced oral spines, and a group of five more widely spaced proximal spines. All spines are long (70-110  $\mu$ m) and arise from an extensive gymnocyst developed proximally and laterally, typically

indenting the very thin cryptocystidean band, which is delimited by a smooth and prominent rim. However, in a possibly teratologic ancestrula, the spines were reduced to only a few in the distal group, with the cryptocystidean band almost entirely absent (Fig. 7G). A hint of denticulation, consisting of about six low and widely spaced denticles, is visible along the deep distal margin of the ancestrula (Fig. 7A, C). In one instance, the ancestrula is regenerated as a miniature autozooid with nearly reversed polarity (Fig. 7H). This form of regeneration is common within the genus *Microporella*. Specifically, in the western Mediterranean, it has been previously observed in *M*.

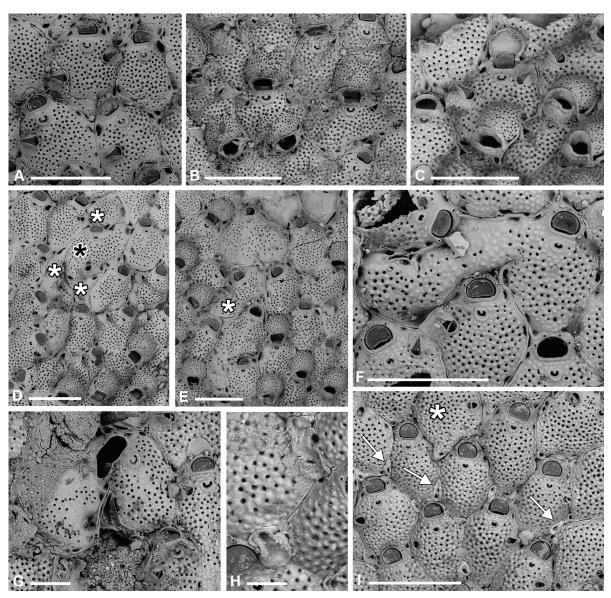


Fig. 9: Microporella browni Harmelin, Ostrovsky, Cáceres-Chamizo & Sanner, 2011. Kenozooids and teratology. All images from colonies found on the lifejacket collected in April 2024 at Spiaggia libera n. 1, Plaia, Catania, except for (H) and (I) found on a buoy stranded at the Simeto River mouth in April 2023. (A) An autozooid apparently with two avicularia as the result of intramural budding following breakage. (B) and (C) Autozooids with two symmetrical avicularia at level with the ascopore (B) or asymmetrical, one shifted proximally (C). (D) and (E) Irregularly shaped autozooids (possibly resulting from fusion processes), one in (D) with a teratological avicularium (just below the black asterisk) and kenozooids (white asterisks), possibly filling areas of convergence between colony lobes. Note the absence of avicularia in almost all autozooids in (E). (F) Large, very irregularly shaped autozooid lacking an avicularium. (G) Fusion of autozooids from two converging lobes producing a shared double orifice with the two halves showing opposite polarities. (H) Small kenozooids arising from marginal areolae. (I) Zone with common small kenozooids (some arrowed) originating from marginal areolae, in one case possibly contributing to the budding of an autozooid (white asterisk). Scale bars: 500 μm: (A), (B), (C), (D), (E), (F), (I); 200 μm: (G); 100 μm: (H).

appendiculata and M. bicollaris (Di Martino & Rosso, 2021). It is worth noting that the exposed gymnocyst of newly metamorphosed ancestrulae with none-to-few already-budded periancestrular autozooids is entire hinting at the need of local resorption processes where budding loci form for the production of further periancestrular autozooids, a condition already observed in ancestrulae of Fenestrulina species (unpublished data) and possibly common to several taxa.

Kenozooids are described for the first time in this species. They are polygonal to irregularly shaped and slightly smaller than autozooids (ca 300-500 μm in maximum dimensions), with the frontal surface only pierced by pseudopores and a few marginal elongate areolae, similar to autozooids (Fig. 9D, E, see white asterisks). Smaller kenozooids (80-140 µm in maximum dimensions) have also been observed, only consisting of a funnel-shaped cryptocystidean shelf surrounding a single, sometimes very wide and irregularly shaped, pseudopore. These kenozooids apparently form from marginal areolae in specific colony zones (Figs 9H, I some arrowed; 10E). Furthermore, in one instance, a marginal areola clearly contributes to the budding of an autozooid (Fig. 9I, see asterisk). Groups of relatively small kenozooids were observed in a colony growing directly on the strap of the lifejacket collected in September 2023. This colony encrusted the net of plastic fibers but grew beneath some partially raised fibers, intertwining with them (Fig. 10A, B). This is evidenced by frontal calcification locally partly covering the fibers (Fig. 10C), deformations, and the apparent crossing of some fibers through the colony (Fig. 10E), sometimes isolated within small kenozooids or even "piercing" and closely overarching vital parts of autozooids such as the orifice (Fig. 10D).

A single closure plate, similar in nature to autozooidal frontal shields, was also observed, sealing the orifice of an autozooid (Fig. 10D).

Abnormal autozooids, interpreted as teratological, were observed in damaged zones and at the contact points between possible lobes/parts of the same colony. They consist of larger and/or irregularly shaped autozooids, possibly resulting from fusions, lacking avicularia (Fig. 9D-G), showing unusually shaped and located avicularia (Fig. 9D, black asterisk), or lacking avicularia but having a double-fused orifice (Fig. 9G).

## Identification of young colonies

When small and infertile colonies of *M. browni* grow in close proximity to young colonies of *M. hastingsae*, as observed in some of our studied substrata (ostreids on the 2024 lifejacket from the Gulf of Catania: Fig. 2D), distinguishing between the two species can be challenging, particularly when ovicells and even avicularia are absent on some/several zooids. Although the morphology of the orifice is a highly diagnostic feature, certain characters of the ancestrula and periancestrular autozooids can also aid in identification, especially when the orifices are covered by opercula. The development of the cryptocystidean band in the ancestrula differs between the two species: in *M. browni*, it is uniformly very narrow, whereas in *M. hastingsae*, it is more extensive proximally. Furthermore, a faint crenulation (though visible only using SEM)

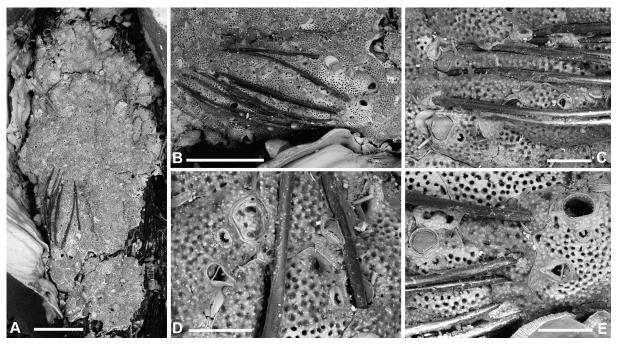


Fig. 10: Microporella browni Harmelin, Ostrovsky, Cáceres-Chamizo & Sanner, 2011. Interaction with plastic fibers. All images from the colony found on the lifejacket collected in September 2023 at Spiaggia libera n. 1, Plaia, Catania. (A) The whole colony on the strap. The basal surface of an oyster encrusting the opposite side of the strap on the backside is also visible. (B) Plastic fibers apparently passing through the colony owing to its growth partly beneath them. (C) Frontal calcification incorporating plastic fibers. (D) Two orifices one closely overarched by a fiber and one covered with a closure plate. (E) Plastic fibers passing through kenozooids. Scale bars: 1 cm: (A); 1 mm: (B), 200 μm: (C), (D), (E).

around the opesial margin of the ancestrula occurs in the former species that is absent in the latter. Pseudopores in the frontal shield also differ, with *M. hastingsae* having fewer but larger and more infundibular pseudopores compared to *M. browni*.

Young colonies of M. ichnusae are also difficult to distinguish, especially from M. hastingsae, due to their similar general appearance when autozooids still lack the two distal avicularia. The main similarities include the frontal shield with fine tubercles and relatively few pores, the orifice with proximolateral condyles lacking distal denticulation, and the initial presence of a frontal, lateral avicularium. However, in M. hastingsae, pseudopores are more infundibular, the single avicularium is comparably smaller and usually located at least at ascopore level, whereas in M. ichnusae the avicularium is larger and usually located at mid-autozooid length. Also, the ancestrula differs slightly between the two species, with M. ichnusae having a more uniformly narrow and flared cryptocystidean band that is more indented by spines, compared to the proximally wider cryptocyst in M. hastingsae.

### **Discussion and Conclusions**

This report marks the second recorded occurrence (Fig. 5, red dots) of *M. hastingsae* outside its originally documented range (Fig. 5, red triangles), i.e., the northern Red Sea and the southern entrance to the Suez Canal (Harmelin *et al.*, 2011). The species was also previously found in Sicily on plastic debris stranded at the mouth of the Simeto River, south of Catania, in April 2023 (Fig. 5, red triangle in Sicily), with a single fertile colony observed (Rosso & Siddiolo, 2024). Subsequent collections in additional selected localities of the northern and eastern coasts of Sicily confirmed the recurring occurrence of this Lessepsian migrant on plastics in the Gulf of Catania and its spread to a wider geographical area, including the southeastern coast of Sicily (Santa Maria del Focallo, Ragusa) (Fig. 1, Table 1).

We also report the second finding of M. browni in the Mediterranean and the first occurrence (Fig. 5, yellow dots, only one reported for the three localities in the Catania area) outside the Levantine Sea (Lebanon: Fig. 5, yellow triangle), where it was initially reported by Harmelin et al. (2011). These authors proposed that M. browni was introduced to the Levantine Sea via shipping through the Suez Canal, with its native distribution centered in the Indian Ocean, from the Oman Sea to the Maldive Islands, including the Gulf of Aden. Colonies that can be confidently ascribed to M. browni were collected on material stranded on the eastern side of the Tyrrhenian Sea at the Capo Milazzo peninsula (Ponente beach); at Torre Faro, close to the Messina Strait, in the northwestern Ionian Sea; and predominantly along a wide coastal sector near Catania (from Aci Castello in the north to the Simeto River mouth in the south), in the western Ionian Sea (Fig. 1, Table 1). As remarked above, many features vary slightly between colonies from different localities, as well as compared to the populations studied by Harmelin et al. (2011), which were considered as potential distinct geographical entities in a cline. Molecular analyses are needed to more confidently determine whether our colonies from Sicily are conspecific with all populations, given the observed independent and inconsistent character variation across geographical locations.

It is noteworthy that while our April 2023 collection yielded only an ovicellate but senescent colony of M. hastingsae, comprising mostly dead autozooids and only some with opercula and avicularian mandibles or cuticles, subsequent collections from December 2023 and April 2024 revealed several living/recently dead colonies, with many ovicells and polypides present in almost all autozooids. Similarly, in April 2023, we found just a single plastic item encrusted by M. browni, with several young colonies and isolated ancestrulae, some having only 1-2 budded autozooids (Fig. 7), making confident identification challenging due to the absence of ovicells. In contrast, the new collections over the past year suggest that both species are now commonly found on floating plastics in wider coastal areas around Sicily. However, based on our documentation, we cannot definitely rule out the prior presence of these species in the area. Indeed, we lack historical data regularly monitoring the occurrence of Microporella species in these specific localities, except for their documented absence from fouling communities on plastic items occasionally collected in various occasions and localities around Sicily (mostly in the Gulf of Catania) and some localities in southern Apulia over time (AR, personal data). The only exceptions were M. modesta and M. appendiculata. The former species occurred with two colonies on marine litter fouling a submerged plastic bag collected in 2012 by scuba diving at 4 m depth in the Ognina marina, northern Gulf of Catania (see Di Martino & Rosso, 2021). The latter species occurred with three colonies on stranded items (an unidentified plastic fragment plus a fleshy alga) collected at Portopalo di Capo Passero in August 2021 (AR, personal data).

Despite constraints due to the limited data available, a recent arrival of M. hastingsae and M. browni in the area seems plausible, with a possible very recent and rapid increase in their abundance on drift plastic debris. For the first discovered colony of M. hastingsae at the Simeto River mouth, Rosso & Siddiolo (2024) suggested that the species might have been introduced to the Mediterranean via the Suez Canal at any time within the last decade, possibly also favoured by its recent enlargement (e.g., Zenetos, 2017). Rosso & Siddiolo (2024) also proposed that the findings in the Ionian Sea could represent the first evidence of an ongoing spread of the species from its first distribution in the northern Red Sea passing through the Levantine Sea. This transfer could have occurred through drift plastics (Fig. 11, path for blue colonies and larvae), either following a single travel event or through multiple "stepping stone" displacements via yet-undiscovered populations in the eastern Mediterranean. A similar scenario is also suggested for M. browni, which has an established presence in Lebanese waters at least since 2002 (Harmelin et al., 2011), but lacks known intermediate

population(s) between its first documented Mediterranean site and the new findings along the Sicilian Ionian coasts. This expansion observed for the two species of Microporella mirrors the pattern seen in the Mediterranean for other non-native bryozoan species such as Smittina nitidissima Hincks, 1880 (see Rosso et al., 2018). The transport of these species via drift plastic items from the Levantine to the Ionian Sea is plausible, supported by water circulation patterns in the eastern Mediterranean (e.g., Pinardi et al., 2015: fig. 5). Encrusting benthic species, especially non-native ones, can foul fixed plastic and other anthropogenic structures including floating debris. In recent times, these have provided new surfaces for colonization and an expanding habitat for rapid proliferation of species as documented across multiple groups of organisms in the Mediterranean and other oceans (e.g., Minchin et al., 2006; Airoldi et al., 2015; Ivkić et al., 2019; Subías-Baratau et al., 2022; Kannan et al., 2023; Giacobbe et al., 2024), supplementing the long-utilized natural drift substrata (e.g., Belmonte, 2019). According to Berline et al. (2021), the general surface circulation of the Ionian Sea is influenced by three main water bodies: one from the west, likely originating from the Sicily Channel, one from the north, probably originating in the northern Ionian Sea and/or the southern Adriatic Sea, and a third, from the south, originating south of the Peloponnese in the Aegean Sea. The provenance of the latter water body from an eastern region close to the Levantine Sea suggests a potential pathway for the transport of plastic items colonized by both *Microporella* species from that area in the eastern Mediterranean. Furthermore, currents alongshore the Ionian coast of Sicily flow southwards from the Messina area (e.g., Bohm et al., 1987; Raffa & Hopkins, 2004). However, the transit of these waters toward the Sicily Strait, and even to easternmost part of the southern Sicilian coast, seems hampered by

the formation of local gyres that persist from seasonal to decadal timescales (e.g., Reyes Suarez *et al.*, 2019).

The fertility of several colonies and the presence of colonies of different sizes, including juveniles and recently metamorphosed ancestrulae, indicate a robust reproductive capability of both M. hastingsae and M. browni, suggesting that these species have established self-sustaining populations on plastic items. It is also plausible that larvae are released from these plastics when they reach coastal zones and last in the water for some time before stranding. Subsequently, larvae could settle on natural substrata available in the area, potentially leading to local establishment and further spread of the species (Fig. 11. blue larvae released from mature colonies on drift plastics potentially colonizing natural habitats). The case of Schizoporella pungens Canu & Bassler, 1928, reported by Winston (2012), exemplifies the role of drift plastic in transferring benthic species capable of thriving in fouling communities associated with such debris. This species, originally from the Caribbean and south Florida, became established in the Indian River area of northeast Florida within a couple of years after being first detected on drift plastic in 2002, and subsequently on fouling panels just one year later.

While this passive long-distance transport on drift plastic could explain the direct arrival of *Microporella* species along the Ionian coast, the presence of drift objects colonized by *M. hastingsae* in the eastern Sicily Strait and of *M. browni* on the western side of the Capo Milazzo Peninsula in the Tyrrhenian Sea is somewhat puzzling. The surface currents in the southern Tyrrhenian Sea are primarily composed of eastward flowing Atlantic Waters (e.g., Iacono *et al.*, 2021: fig. 3), and the connectivity between the Ionian Sea and the Tyrrhenian Sea is minimal (e.g., Celentano *et al.*, 2020: fig. 2), which complicates the direct transfer between these sub-basins of the Mediterranean. A similar challenge exists for alongshore transfer from the Ionian

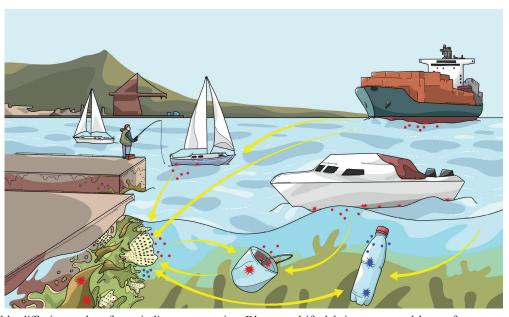


Fig. 11: Possible diffusion paths of non-indigenous species. Blue: on drift debris transported by surface currents, potentially releasing larvae colonizing anthropogenic fixed installations and/or natural habitats; red: on long-distance commercial vessels spreading to harbours and recreational boats in marinas. Star-shaped patches indicate colonies; dots are for short-living larvae; arrows: possible paths. See text for further explanation. Illustration by Petter Nordenhaug (University of Oslo, Norway).

Sea to Sicily Strait (see above).

These considerations suggest that at least some of the analysed fouling communities, particularly those in the Tyrrhenian Sea, may be sustained by larvae of different origin, implying different and/or additional vectors and pathways to the transport provided by the surface circulation of the water bodies. It is possible that plastics could be locally colonized by larvae released from colonies occurring on boats (Fig. 11, path for red colonies and larvae). Several ports and marinas occur all along the coast of Sicily, including a major commercial port near the beach in Milazzo where M. browni was found. Similarly, important commercial and touristic ports are located in the southeast coast of Sicily, an area directly connected through trade routes to ports at the eastern Mediterranean and the Suez Canal (Ship Traffic Density Map of Mediterranean Sea, 2014). Ports, especially those frequented by tourist boats, experience heavy maritime traffic and usually host a number of NIS (Ulman et al., 2017; Tempesti et al., 2020; 2022; Xavier et al., 2021). Whereas main commercial ports harbouring long-distance boats may act as primary hubs for the introduction of non-native species, recreational boating has been identified as a key vector for their local transfer (e.g., Ferrario et al., 2017; 2024; Castro et al., 2022). A similar mechanism of spread has been envisaged for Catenicella paradoxa Rosso, 2009 (see Mytilineou et al., 2016). This species, now considered cryptogenic (after considerations in Rosso, 2009), spread from Capo Passero Isle, at the southeastern tip of Sicily, northwards to the Plemmirio Marine Protected Area (near Siracusa) and the Ciclopi Isles Marine Protected Area (near Catania). This countercurrent spread is consistent with the timeline of sample collection from 2006 at Capo Passero to 2014 and 2015 at Siracusa and Catania, areas situated progressively northwards and that were previously investigated but unsuccessfully for C. paradoxa (Rosso et al., 2013; 2018; 2019). Local shipping could similarly help explain the transit of the Microporella species considered here from the Ionian to the Tyrrhenian Sea in the north and to the Sicily Strait to the south. As Ferrario et al. (2020) reported, such "anthropogenic activities can increase connectivity among even distant localities, breaking geographical barriers". In this scenario, the stranded plastic items may be colonized secondarily at a local level directly from larvae released from colonies on the boats or assuming an intermediate transit of species to local anthropogenic structures (e.g., pontoons) in ports and/or marinas (Fig. 11, paths linking red colonies and larvae). To fully support this hypothesis, it would be crucial to investigating the potential presence of our target Microporella species within ports or marinas along the coasts of Sicily, as well as on boats moored in these ports. Organizing localized surveys and monitoring NNS in these contexts, preferably within large focused projects, is urgently needed to provide baseline data on their presence and abundance. Citizen science could also play an important role in this effort, particularly through the involvement of recreational divers. This approach has been successful in studies focusing on more conspicuous species (e.g., Kleitou et al., 2019; Zenetos et al., 2013;

2024; authors' unpublished data) but could also offer valuable insights for bryozoans, a group for which citizen science efforts are still rare, except for notable examples such as the Doris portal initiative (https://doris.ffessm. fr) and a related book (André et al., 2014). To date, and to the best of our knowledge, only a few marinas (i.e., Palermo on the Tyrrhenian side, Riposto, Siracusa and Marzamemi along the Ionian side, and Marina di Ragusa and Licata along the Sicily Strait) have been surveyed for non-indigenous bryozoans by Ulman et al. (2017). However, none of these marinas have yielded colonies of M. hastingsae or M. browni. Given that these surveys were conducted a few years ago (2016), and considering how rapidly marine communities can change -evidenced by the transition from finding only one partially dead colony in April 2023 to many vibrant colonies by late 2023 and early 2024—it is likely that these species were simply not present at the time of the previous surveys but may likely be now established in these areas. However, local populations of M. hastingsae and M. browni need to be discovered and documented in Sicily before confirming the establishment of these two species in the region, in accordance with guidelines for monitoring the spread of NIS (e.g., Marchini et al., 2015; Zenetos et al., 2022b), as discussed in Rosso & Siddiolo (2024).

Notably, unlike the three species considered native to the Mediterranean (see Rosso & Di Martino, 2023), M. hastingsae and M. browni were very frequently found on stranded plastic items, usually with several colonies (Table 1). The abundance/dominance of non-native species on anthropogenic items, especially large fixed structures and drift plastic, is well known deriving from their opportunistic behavior and high ecological flexibility, which makes them more tolerant to variable environmental conditions, more resilient to disturbances, and more competitive compared to native species (e.g., Canning-Clode et al., 2013; Ferrario et al., 2020; Giacobbe et al., 2024). Among the three native *Microporella* species, only *M*. appendiculata and M. modesta were previously documented, though occasionally, in fouling communities on drift materials, especially plastic. Notably, both species are among the most widespread in the Mediterranean and have been repeatedly recorded around Sicily. They are well adapted to a variety of shelf habitats, also showing a certain tolerance to high light intensity, being sometimes associated with infralittoral algae (e.g., Rosso et al., 2019) and the leaf layer in *Posidonia* meadows (see Di Martino & Rosso, 2021), respectively. The finding of M. ichnusae is particularly significant, as this species, previously known only from a few colonies collected in caves and overhangs in northwest Sardinia, may have a wider distribution within the same ecoregion (i.e., the western Mediterranean) than previously understood.

Given the current context of climate change and the rapid increase in marine water temperatures, it is expected that the two non-native Lessepsian species, *M. hastingsae* and *M. browni*, which are native to the Red Sea and the Indo-Pacific, will increasingly be favoured. They may rapidly spread westward and northward, contributing to the tropicalization of the Mediterranean biota.

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